virion production. Relocation of the p5 promoter to the 3' end of the *cap* gene (pW1909) resulted in the lowest expression of the long forms of Rep protein, the highest expression of the short forms of Rep protein, and the highest production of recombinant virions. Introduction of an efficient polyadenylation site (pRCM.globinpolyA) between the transcriptional start site and the first codon in the coding sequence of the long forms of Rep protein also decreased expression of the long forms of Rep and increased expression of the short forms of Rep protein, but less so. Consequently, recombinant virion production was improved relative to the pRCM but was less than pW1909. Modification of the Kozak sequence so as to reduce translation initiation efficiency (pRCM.kozak) and introduction of a sub-optimal polyadenylation site had little effect on Rep protein expression and resulted in little improvement in recombinant virion production.

In summary, the AAV helper function systems, host cells, and methods of the present invention allow for high-efficiency production of rAAV virions by reducing the amount of the long forms of Rep proteins produced. Here, we show that AAV helper function vectors that produce only small amounts of the long forms of Rep protein provide for higher titer rAAV virion production.

The invention may be embodied in other specific forms without departing from its essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes that come within the meaning and range of equivalency of the claims are to be embraced within their scope.

We claim:

1. A method of producing recombinant AAV virions, comprising the steps of:

(a) introducing an AAV vector into a host cell;

(b) introducing an AAV helper function vector comprising an AAV *rep* coding region into the host cell, wherein said *rep* coding region comprises a nucleotide sequence coding for long forms of Rep protein and short forms of Rep protein, such that the host cell produces greater quantities of the short forms of Rep protein than of the long forms of Rep protein;

(c) expressing accessory functions in the host cell; and

(d) culturing the host cell to produce recombinant AAV virions.

2. A recombinant AAV virion produced according to the method of claim 1.

3. The method of claim 1, wherein the AAV helper function vector further comprises an AAV *cap* coding region.

4. The method of claim 1, wherein the host cell produces at least ten-fold greater quantities of the short forms of Rep protein than of the long forms of Rep protein.

5. A host cell comprising an AAV helper function vector, said AAV helper function vector comprising an AAV *rep* coding region, wherein the *rep* coding region comprises a nucleotide sequence that codes for long forms of Rep protein and short forms of Rep protein, such that the host cell produces greater quantities of the short forms of Rep protein than of the long forms of Rep protein.

6. The host cell of claim 5, wherein the AAV helper function vector further comprises an AAV *cap* coding region.

7. A method of producing recombinant AAV virions, comprising the steps of:

(a) introducing an AAV vector into a host cell;

(b) introducing an AAV helper function vector comprising an AAV *rep* coding region into the host cell, wherein said *rep* coding region comprises a nucleotide sequence coding for long forms of Rep protein and short forms of Rep protein, and said AAV helper function vector causes the host cell to produce an amount of the long forms of Rep protein that is substantially less than an amount produced from an AAV helper function vector expressing the long forms of Rep protein under control of an AAV p5 promoter;

(c) expressing accessory functions in the host cell; and

(d) culturing the host cell to produce recombinant AAV virions.

8. A recombinant AAV virion produced according to the method of claim 7.

9. The method of claim 7, wherein the AAV helper function vector further comprises an AAV *cap* coding region.

10. A host cell comprising an AAV helper function vector, said AAV helper function vector comprising an AAV *rep* coding region, wherein the *rep* coding region comprises a nucleotide sequence that codes for long forms of Rep protein and short forms of Rep protein, and said AAV helper function vector causes the host cell to produce an amount of the long forms of Rep protein

that is substantially less than an amount produced from an AAV helper function vector expressing the long forms of Rep protein under control of an AAV p5 promoter.

11. The host cell of claim 10, wherein the AAV helper function vector further comprises an AAV *cap* coding region.

12. A method of producing recombinant AAV virions, comprising the steps of:

(a) introducing an AAV vector into a host cell;

(b) introducing an AAV helper function vector comprising AAV *rep* and *cap* coding regions into the host cell to express Rep and Cap gene products, wherein the *rep* coding region is under the control of an inducible promoter that expresses an amount of *rep* RNA that is substantially less than an amount expressed from an AAV p5 promoter;

(c) expressing accessory functions in the host cell; and

(d) culturing the host cell to produce recombinant AAV virions.

13. A recombinant AAV virion produced according to the method of claim 12.

14. A method of producing recombinant AAV virions, comprising the steps of :

(a) introducing an AAV vector into a host cell;

(b) introducing an AAV helper construct comprising AAV *rep* and *cap* coding regions into the host cell to express Rep and Cap gene products, said *rep* coding region comprising a nucleotide sequence coding for long forms of Rep protein and short forms of Rep protein, wherein

the *rep* coding region is regulated by an inducible promoter such that the host cell produces greater quantities of the short forms of Rep protein than of the long forms of Rep protein;

(c) expressing accessory functions in the host cell; and

(d) culturing the host cell to produce recombinant AAV virions.

15. A recombinant AAV virion produced according to the method of claim 14.

16. The method of claim 14, wherein the host cell produces at least ten-fold greater quantities of the short forms of Rep protein than of the long forms of Rep protein.